

IN THE CLAIMS

Please amend the claims under the provisions of 37 CFR §1.121(a)(2)(ii), so that they appear as follows:

1. (CURRENTLY AMENDED) [[A]] In a method for the coamplification of two or more target nucleic acids having different sequence compositions, and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids, or their primer extension products, wherein each of the two or more target nucleic acids present in said reaction mixture is present in said mixture at comparable copy number with respect to other target nucleic acid(s) that is not more than 10-fold different from the copy number of any other of the two or more target nucleic acids, and a ~~hot start~~ DNA polymerase at a first temperature, T_1 , for denaturation of the strands of the target nucleic acids or their primer extension products, ~~wherein said hot start DNA polymerase is activated at T_1 , and~~

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2 , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3 , provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same,

~~wherein the reaction mixture in at least one of the primary amplification cycles further comprises from 1 to 20 weight % of a nonionic polymeric volume exclusion agent, and~~

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more primer extension products having different sequence compositions as an indication of coamplification of the target nucleic acids, the improvement comprising,

using as the DNA polymerase included in the reaction mixture of step (A), a chemically-modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50°C, and

using, in at least one of the primary amplification cycles, 1 to 20 weight % of a nonionic polymeric volume exclusion agent.

2. (CURRENTLY AMENDED) [[A]] In a method for the coamplification of two or more target nucleic acids having different sequence compositions, and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids, or their primer extension products, wherein each of the two or more target nucleic acids present in said reaction mixture is present in said mixture at comparable copy number with respect to other target nucleic acid(s) that is not more than 10-fold different from the copy number of any other of the two or more target nucleic acids, and a ~~hot-start~~ DNA polymerase, at a first temperature, T_1 , for denaturation of the strands of the target nucleic acids or their primer extension products, ~~wherein said hot start DNA polymerase is activated at T_1 , and~~

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2 , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3 , provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same,

wherein the reaction mixture in at least one of the primary amplification cycles further comprises ~~from 1 to 20 weight % of a nonionic, polymeric volume exclusion agent, and~~ a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization, and,

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more primer extension products having different sequence compositions as an indication of coamplification of the target nucleic acids, the improvement comprising,

using as the DNA polymerase included in the reaction mixture of step (A), a chemically-modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50°C, and
using, in at least one of the primary amplification cycles, 1 to 20 weight % of a nonionic polymeric volume exclusion agent.

3. (CANCELED)

4. (CURRENTLY AMENDED) ~~[[A]]~~ In a method for the coamplification of two or more target nucleic acids having different sequence compositions, and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids, or their primer extension products, wherein each of the two or more target nucleic acids present in said reaction mixture is present in said mixture at comparable copy number with respect to other target nucleic acid(s) that is not more than 10-fold different from the copy number of any other of the two or more target nucleic acids, and a ~~hot-start~~ DNA polymerase, at a first temperature, T_1 , for denaturation of the strands of the target nucleic acids or their primer extension products, ~~wherein said hot start DNA polymerase is activated at T_1 , and~~

(B) priming the denatured strands with a set of primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2 , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3 , provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same, and

wherein the reaction mixture in at least one of the primary amplification cycles ~~further~~ optionally comprises ~~from 1 to 20 weight % of a nonionic, polymeric volume exclusion agent, and~~ optionally a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridisation, and,

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the

last primary amplification cycle, detecting two or more primer extension products having different sequence compositions as an indication of coamplification of the target nucleic acids, the improvement comprising,
using as the DNA polymerase included in the reaction mixture of step (A), a chemically-
modified thermostable hot start DNA polymerase enzyme that becomes active only after
incubation at temperatures above 50°C, and
using, in at least one of the primary amplification cycles, 1 to 20 weight % of a nonionic
polymeric volume exclusion agent.

5. (CURRENTLY AMENDED) A method according to one of claims [[1 – 4]] 1, 2, or 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate.

6. (PREVIOUSLY PRESENTED) A method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula:



wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000.

7. (PREVIOUSLY PRESENTED) The method according to claim 6, characterized in that R may represent 1,2-ethylene, 1,3-propylene, 1,2-propylene, 2-hydroxy-1,3-propylene, 3-hydroxy-1,2-propylene, 1,4-butylene, 1,3-butylene, or 1,2-hexylene.

8. (PREVIOUSLY PRESENTED) The method according to claim 6, characterized in that the polyether is poly(ethylene glycol).

9. (PREVIOUSLY PRESENTED) The method according to claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 1000 daltons to 2,000,000 daltons.

10. (PREVIOUSLY PRESENTED) The method according to Claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 daltons to 500,000 daltons.

11. (PREVIOUSLY PRESENTED) The method according to Claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000 daltons.

12. (PREVIOUSLY PRESENTED) The method according to claim 5, characterized in that the volume exclusion reagent is a dextran.

13. (PREVIOUSLY PRESENTED) The method according to claim 12, characterized in that the dextran has a molecular weight in the range of 1000 daltons to 2,000,000 daltons.

14. (PREVIOUSLY PRESENTED) The method according to Claim 12, characterized in that the dextran has a molecular weight in the range of 3000 daltons to 500,000 daltons.

15. (PREVIOUSLY PRESENTED) The method according to Claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 daltons to 60,000 daltons.

16. (PREVIOUSLY PRESENTED) The method according to claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-dihydroxypropyl acrylate).

17. (WITHDRAWN) An amplification reaction composition for performing the method of Claim 1, wherein said composition is buffered to a pH of from about 7.5 to about 9, and wherein said composition comprises:
one or more sets of primers,
a thermostable hot-start-DNA polymerase,
a plurality of dNTP's, and
1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, and
optionally a probe.

18. (WITHDRAWN) The amplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 15 weight % of a nonionic, polymeric volume exclusion agent.

19. (WITHDRAWN) The amplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent.

20. (WITHDRAWN) A kit for the coamplification of two or more target nucleic acids according to the method of Claim 1, comprising:

(a) an amplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:

one or more sets of primers,

a thermostable hot-start DNA polymerase,

a plurality of dNTP's, and

1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a nonionic, polymeric volume exclusion agent, and

(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble substrate.

21. (WITHDRAWN) A self-contained test device for performing the amplification method of Claim 1, comprising, in separate compartments:

(a) an amplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:

one or more sets of primers,

a thermostable hot-start DNA polymerase,

a plurality of dNTP's, and

1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a nonionic, polymeric volume exclusion agent, and,

(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble substrate, the compartments being connected in the test device so that the amplification reaction composition can be brought into contact with the capture reagent after amplification without

opening the test device.

22. (WITHDRAWN) A kit for preparing an amplification reaction composition according to claim 17 comprising:
at least one hot-start DNA polymerase, and
at least one polymeric exclusion reagent.

23. (CURRENTLY AMENDED) The method according to Claim 1, 2, [[3,]] or 4, wherein the volume exclusion agent is present in said reaction mixture in a concentration of 1-15 weight %.

24. (CURRENTLY AMENDED) The method according to Claims 1, 2, [[3,]] or 4, wherein the volume exclusion agent is present in said reaction mixture in a concentration of 1-8 weight %.

25. (NEW) The method according to Claims 1, 2, or 4, wherein said chemically-modified DNA polymerase is modified by reaction with an aldehyde.